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# *Saprolegnia parasitica* zoospore activity and host survival indicates isolate variation in host preference

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**Abstract**

The ubiquitous freshwater pathogen *Saprolegnia parasitica* has long been considered a true generalist, capable of infecting a wide range of fish species. It remains unclear, however, whether different isolates of this pathogen, obtained from distinct geographic locations and host species, display differences in host preference. To assess this, the current study examined the induced zoospore encystment responses of four *S. parasitica* isolates towards the skin of four fish species. While three of the isolates displayed 'specialist' responses, one appeared to be more of a 'generalist'. *In vivo* challenge infections involving salmon and sea trout with the 'generalist' (salmon isolate EA001) and a 'specialist' (sea trout isolate EA016) pathogen, however, did not support the *in vitro* findings, with no apparent host preference reflected in infection outcomes. Survival of sea trout and salmon though was significantly different following a challenge infection with the sea trout (EA016) isolate. These results indicate that while *S. parasitica* isolates can be considered true generalists, they may target hosts to which they have been more frequently exposed (potential local adaptation). Understanding host preference of this pathogen could aid our understanding of infection epidemics and help with the development of fish management procedures.

**Keywords:** host preference; specialist; generalist; local adaptation; oomycete.

Declarations of interest: none.

## Introduction

Host specificity is an important parasite trait; providing an accurate depiction of a parasite's ecological niche (Poulin and Mouillot 2003). It is determined by the number of host species that a parasite can successfully invade and the taxonomic relationship between these host species. While some parasites might trade off their virulence (the severity of infection) against transmissibility (ability to spread infection from host to host) with optimum parasite fitness striking a balance between the production of transmission stages and damage to the host, for others the interaction is more complex (Acevdeo et al. 2019). This is partly driven by host range; a given parasite species may infect a wide range of phylogenetically distinct host taxa, with parasite fitness varying from host to host. Thus, the composition of the host population presents a selective pressure that contributes to evolution of parasite generalism or specialism (Futuyma and Moreno 1988). Parasites with low host specificity are considered generalists, capable of switching between distantly related host species, moreover they tend to exhibit a similar level of virulence across their broad host range (Poulin and Mouillot 2003; Leggett et al. 2013). More specialised parasites may possess a high specificity for certain host species or taxonomic group for which they exhibit an optimal level of virulence.

Fungal and fungal-like parasites are thought to possess the broadest host range of any parasite group (Fisher et al. 2012). Perhaps the most notorious example being the aquatic

chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) that infects over 700 amphibian species (Olson et al. 2013) and is also capable of infecting fish (Liew et al. 2017). Furthermore, several fish pathogenic oomycetes are considered true generalists able to infect several different host families (Gozlan et al. 2014). Members of the *Saprolegnia* genus are particularly destructive; *Saprolegnia diclina* is a virulent pathogen of fish eggs (Kitancharoen et al. 1997; Fregeneda-Grandes et al. 2007; van den Berg et al. 2013) and *S. ferax* is believed to be partly responsible for declines in amphibian populations (Kiesecker et al. 2001; Pounds 2001). *S. parasitica* is arguably the most important animal pathogenic oomycete with a reported 1 in 10 of all farmed raised salmon succumbing to saprolegniasis and frequent associations with declining natural wild fish populations (van West 2006). Previously, *S. parasitica* was considered opportunistic, only able to infect fish hosts as a secondary pathogen. Several salmonid studies, however, have highlighted that certain isolates of *S. parasitica* are primary invaders and highly virulent (Neish 1977; Willoughby and Pickering 1977). Despite this, marked differences in virulence have been observed between *S. parasitica* isolates (Yuasa and Hatai 1995) and the host range of individual isolates remains unexplored.

*S. parasitica* produces free-swimming zoospores during the infective stage of its life cycle. These zoospores are unicellular, single nucleated cells that are able to swim freely via two flagella; one tinsellated and one whiplash flagellum (Burr and Beakes 1994). They are responsible for the first essential step in establishing an infection, namely locating and attaching to a host. Upon achieving this, these infectious propagules encyst, germinate and sprout mycelial hyphae which then penetrate the host tissues (Willoughby et al. 1983; Diéguez-Uribeondo et al. 1994). If isolates of *S. parasitica* are specialised for particular hosts, this could be reflected in their induced zoospore encystment responses towards different fish species.

The current study combines *in vitro* induced zoospore encystment data with targeted *in vivo* challenge experiments to uncover the extent of specialism/generalism within this species.

The *in vitro* investigations assessed the host preference of four *S. parasitica* isolates that are both geographically distinct and originally isolated from different host species. The *in vivo* studies aimed to determine whether the *in vitro* findings were reflected in challenge infection outcomes. Moreover, infections involving different populations of the same species were included to determine whether *S. parasitica* host preference exists at a population level.

We hypothesise that while the isolates investigated may not be highly specialised to a single host species, they do display preferences towards a limited number of host species. A phylogenetic analysis of the four *S. parasitica* isolates based on nuclear ribosomal internal transcribed spacer (nrITS) sequence data has also been included to examine their position within the *Saprolegnia* taxonomic system proposed by Sandoval-Sierra et al. (2014). This data could potentially expand our understanding of *S. parasitica* infections and inform future aquaculture practices.

## Materials and Methods

### *Host origin and maintenance*

Atlantic salmon (*Salmo salar),* sea trout (*Salmo trutta),* common carp (*Cyprinus carpio*) and three-spined stickleback (*Gasterosteus aculeatus*) (n=6 per species) were net caught from hatcheries or the wild and delivered to our aquarium facilities at Cardiff University for use in the induced encystment assays (see Table 1). Moreover, further net-caught Atlantic salmon and sea trout (n=60 per species) were obtained for use in the experimental challenge infections (see Table 1). Prior to experimental procedures, fish were maintained in 90 L tanks at a density of 1 fish $L^{-1}$. Both prior to and during experimental procedures, fish were exposed to a water temperature of 12±0.5°C, oxygen saturation of >91%, 12 h light: 12 h dark cycle and fed trout pellets daily unless otherwise stated.

95 Saprolegnia *culture and zoospore production*

96 Four *Saprolegnia parasitica* isolates were obtained directly from four naturally infected fish
97 hosts collected during routine sampling by the Environment Agency (see Table 2). On the
98 riverbank, a small mycelial clump (approx. 4-5 $cm^2$) was extracted from the affected tissue of
99 a live, recently caught fish using forceps and placed immediately onto a potato dextrose agar
100 (PDA, 39g $L^{-1}$) plate. The plate was sealed using parafilm and sent to our facilities at Cardiff.
101 Cultures were sub-cultured monthly onto fresh PDA plates according to Stewart et al. (2017).
102 For zoospore production, petri dishes containing ~40 ml glucose-yeast broth (Glucose 10g $L^{-1}$,
103 Yeast Extract 2.5g $L^{-1}$) were inoculated with three 5 mm diameter plugs of heathy white
104 mycelia from the stock culture. The *S. parasitica* mycelia were left to grow for 72 h at 20°C,
105 then washed with dechlorinated water in order to remove excess glucose-yeast broth. To induce
106 zoospore production, the mycelia were placed in a 50/50 mixture of dechlorinated water and
107 aquarium water at 10°C for 72 h. The resulting zoospore suspension was concentrated via
108 centrifugation at 4600 rpm for 10 min at room temperature. Zoospores in the concentrated
109 suspension were enumerated using a haemocytometer.

110 *DNA extraction and sequencing of internal transcribed spacer (ITS) rDNA*

111 DNA was extracted using the following modified protocol outlined by Vilgalys and Hester
112 (1990). Briefly, ~0.3g of mycelia from each of the respective *S. parasitica* isolates was ground
113 under liquid nitrogen and suspended in 500 µl of 2X (w/v) CTAB extraction buffer (100 mM
114 Tris, 20 mM $Na_2EDTA$, 1.4M NaCl, pH 8.0). The samples were then subject to a freeze-thaw
115 step in which they were placed at -80°C for 10 min and subsequently incubated at 65°C for 30
116 min. Samples were extracted twice using equal volumes of chloroform-isoamyl alcohol (24:1);
117 DNA was precipitated by adding 2 volumes of isopropyl alcohol and placing the samples at -
118 20°C for 24 h. The resulting genomic DNA pellets were washed once with 70% EtOH, dried

under a laminar flow hood and re-suspended in 50 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

PCR amplification of the ITS region was performed using the universal fungal primers of White et al. (1990): 5'-GGAAGTAAAAGTCGTAACAAGG-3' (ITS 5-Forward) and 5'-TCCTCCGCTTATTGATATGC-3' (ITS 4-Reverse). The PCR reaction mix consisted of 15 µl of Taq PCR Master Mix (Qiagen), 1.5 µl of each forward and reverse primer, 20-50 ng genomic DNA and nuclease-free water to give a total reaction volume of 30 µl. The PCR protocol was as follows: initial denaturation at 94°C for 5 min, 5 amplification cycles of: denaturation at 94°C for 30s, annealing at 58°C for 30s and extension at 72°C for 1 min; subsequently a further 33 cycles were included where the annealing temperature was changed to 48°C. A final extension at 72°C for 10 min concluded the PCR. In order to check the PCR products were the correct size (approx. 700 bp), they were run on a 1% agarose gel and visualised using UV-transillumination. PCR products were then sequenced via Sanger sequencing and a NCBI BLAST search for related sequences was used to confirm each sample as *S. parasitica* (>98% sequence identity to *S. parasitica*).

*Phylogenetic analysis*

ITS sequence data for the four *S. parasitica* isolates (see Supplementary materials) investigated here were included in a phylogenetic analysis alongside *Saprolegnia* spp. sequences from GenBank that have been previously designated into phylogenetic clusters by Sandoval-Sierra et al. (2014) and an isolate of *Aphanomyces astaci* served as an outgroup (Supplementary Table S1). The Molecular Evolutionary Genetics Analysis (MEGA) software v10.0.2 was used to first align the ITS sequences using the ClustalW algorithm with default settings, and subsequently construct a phylogenetic tree using the Maximum Likelihood method based on the Jukes-Cantor model. Relative branch support of the tree was estimated using a bootstrap

analysis with 1000 replicates, all other settings were set to default. The tree was converted into Newick format and imported into FigTree v1.4.4 to produce a circular phylogenetic tree.

### In vitro *induced zoospore encystment assays*

To obtain skin samples for the induced zoospore encystment assays, Atlantic salmon, sea trout, common carp and three-spined stickleback (n=6 per species; details in Table 1) were euthanised with an overdose of MS-222 and pithed to destroy the brain (Home Office Schedule 1 method). Skin was subsequently removed from each fish and samples from each respective fish species were pooled together and homogenised in phosphate buffered saline (PBS 1X) at a concentration of 0.1g skin $ml^{-1}$. The homogenised solution was centrifuged at 1000 rpm for 5 min. The resulting pellet was discarded and the supernatant was aliquoted and stored at -20°C until required.

A modified capillary root model (Halsall 1976) was used to assess the induced zoospore encystment responses of the four *S. parasitica* isolates listed in Table 2. The assay was performed in a plastic petri dish (48 mm base diameter x 12 mm depth) containing 5 ml of zoospore suspension prepared as described previously and adjusted to a concentration of ~300 zoospores $ml^{-1}$. A cell scraper was used to ensure no spores were encysted on the sides or base of the petri dishes and that the spores were evenly dispersed throughout the suspension. Two micro-capillary tubes (Drummond Scientific, 2.9 cm length, 20 µl volume) were introduced into the petri dish, containing either the fish skin solution ('test' solution) or PBS (1X 'control' solution) and positioned at specific distances apart using forceps (see Fig. 1A)**Error! Reference source not found.**~~Error: Reference source not found~~. The test and control tubes were left in the zoospore solution for 10 min, then removed and the contents of each expelled into an Eppendorf tube. These tubes were vortexed for 45 s, causing any zoospores to encyst. The number of encysted zoospores in each tube was then counted using a haemocytometer.

To account for potential differences between batches of zoospores, the assays were conducted in the following manner; for each *S. parasitica* isolate two batches of zoospores were produced. Per batch, 10 replicates of the assay were performed against each fish species. Hence, across the two batches of spores per isolate, a total of 20 replicates were achieved against each fish species. To control for potential side bias, the position of the test and control tubes was alternated. Room lighting (constant overhead LED lighting) and temperature (20±1°C) were kept constant throughout the assays. The induced zoospore encystment responses were expressed by a ratio that was calculated for each assay replicate using the following equation:

$$\text{Induced zoospore encystment ratio (IZER)} = \frac{\text{Mean no. of zoospores in 'test' tube}}{\text{Mean no. of zoospores in 'control' tubes}}$$

The assay was subsequently modified to assess the induced zoospore encystment responses of the salmon (EA001) and sea trout (EA016) *S. parasitica* isolates (see Table 2) when presented with a direct choice between the skins of two fish species. Micro-capillary tubes containing salmon skin solution ('salmon test') and sea trout skin solution ('sea trout test') were used in the same assay alongside a PBS control (**Error! Reference source not found.**~~Error: Reference source not found~~Fig. 1B). All other experimental conditions were consistent with those described previously. The induced zoospore encystment responses of these isolates towards salmon and sea trout were calculated using the induced zoospore encystment ratio (IZER) equation above.

In vivo Saprolegnia parasitica *challenge infections*

Challenge infections were conducted to assess whether the induced zoospore encystment ratios (IZERs) obtained for the salmon (EA001) and sea trout (EA016) *S. parasitica* isolates were reflected in infection outcomes. The experimental procedure described here was performed

191 separately for these isolates in order to avoid cross contamination. Zoospore suspensions were
192 prepared as described above and a concentration of ~$3x10^5$ $L^{-1}$ used for all of the experimental
193 infections. Juvenile salmon and sea trout (n=60 per species, see Table 1) were subjected to an
194 adjusted 'ami-momi' technique (Hatai and Hoshiai, 1994) in which they were individually
195 shaken in a net for 30 s to introduce abrasions to the fish body and remove protective mucus.
196 Of these fish, n=50 per species were assigned to the 'treatment' condition and placed into glass
197 aquaria (31 W x 61 D x 31 H cm) separated by species, containing a well-oxygenated zoospore
198 suspension at a density of 1 fish $L^{-1}$ for 24 h without food. Following zoospore exposure, fish
199 were transferred into individual transparent plastic 1 L containers of dechlorinated water and
200 daily feeding was resumed. The remaining fish acted as the 'controls' (n=10 per species) and
201 were handled in exactly the same manner without exposure to zoospores, before transfer to
202 individual containers. Water in both the treatment and control containers was changed every
203 24 h. Fish were checked hourly for signs of saprolegniasis over the duration of the experimental
204 period of 168 h. Fish were categorised as either symptomatic (mild and cleared or severe) or
205 asymptomatic according to the severity of their symptoms (Table 3). Any fish displaying severe
206 signs of infection were euthanised via overdose with MS-222 and pithing.

207 This experiment was subsequently repeated using two different stocks of juvenile
208 salmon from the River Tyne: one from the North Tyne and another from the South Tyne. While
209 there is no available data to confirm whether these were genetically distinct populations of fish,
210 the literature on within-river genetic structure of salmon suggests that river tributaries generally
211 contain distinct populations (Vähä et al. 2007). Hence these will be referred to as different
212 populations from here onwards. The fish were kept separated according to population and all
213 other experimental procedures were the same as those described previously.

*Animal Ethics*

All procedures and protocols followed ARRIVE guidelines, were approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office license PPL 30/3424.

*Statistical Analyses*

Analyses were conducted using R statistical software (version 3.5.1, R Core Team 2018) with the significance threshold $P<0.05$ used for all models. Non-significant terms were removed during model refinement based on Analysis of Variance (Crawley 2007) while model robustness was assessed using residual plots (Pinheiro and Bates 2000).

A Generalised Linear Model (GLM) fitted with a Gaussian error family and identity link function was used to examine the induced zoospore encystment of four *S. parasitica* isolates towards the skin of four fish species. Induced zoospore encystment ratio (IZER) was the dependent term in the model, fixed terms included: *S. parasitica* isolate (EA001, EA016, EA012, CF006), fish skin (salmon, sea trout, common carp, three-spined stickleback - herein referred to as stickleback) and the interaction between these two terms. Zoospore batch (1, 2) was included as a fixed factor in the original model but was removed during model refinement due to non-significance ($P>0.05$). *Post-hoc* analysis (lsmeans package; Lenth 2016) was conducted to compare the IZERs of each *S. parasitica* isolate.

A second GLM fitted with a Gaussian error family and identity link function was used to examine the induced zoospore encystment of the salmon (EA001) and sea trout (EA016) isolates when presented with salmon and sea trout skin in a single assay. The dependent and independent terms were the same as those outlined in the previous model and again, zoospore batch (1, 2) was included in the original model but removed due to non-significance ($P>0.05$).

Comparisons between the IZERs of the two *S. parasitica* isolates were assessed using *post-hoc* analysis (lsmeans package; Lenth 2016).

Proportional odds logistic regression (POLR) models (MASS package; Venables and Ripley 2002) were used to examine the infection outcomes of the *S. parasitica* experimental challenge infections. Infection outcome of each fish (asymptomatic, mild and cleared, severe) was the dependent term in the models, fixed effects included; *S. parasitica* isolate (salmon isolate EA001, sea trout isolate EA016), and fish species/population (model 1 – salmon, sea trout; model 2 – North Tyne, South Tyne salmon). Fish standard length (mm) was also initially included in the models but was found to be non-significant ($P>0.05$) and was consequently removed.

Kaplan-Meier survival plots (survival package; Therneau 2015) were generated for challenge infections involving the sea trout isolate (EA016) only, log-rank tests were used to compare the survival plots. Survival analysis was not possible for the salmon isolate (EA001) as no mortalities occurred during the challenge infections with this isolate.

## Results

### *Phylogenetic analysis*

All four isolates of *S. parasitica* used in the current study fell within Cluster 3 of the taxonomic system proposed by Sandoval-Sierra et al. (2014) (Supplementary Fig. S1)**Error! Reference source not found.**~~Error: Reference source not found~~. Although, identical by ITS, subsequent genome resequencing revealed significant genetic differences between isolates (Matthews 2020).

In vitro *induced zoospore encystment assays*

Induced zoospore encystment ratios (IZERs) displayed by the *S. parasitica* isolates (EA001, EA016, EA012, CF006) were dependent on fish skin (salmon, sea trout, common carp, stickleback) (GLM; $df$=9, $P$<0.0001), but there was no clear trend in preference. The salmon isolate (EA001) showed a significant preference for salmonid and carp over stickleback (Fig. 2A).**Error! Reference source not found.**~~Error: Reference source not found~~ The sea trout isolate (EA016) IZERs were significantly higher for sea trout and common carp compared to salmon or stickleback skin (**Error! Reference source not found.**~~Error: Reference source not found~~Fig. 2B). The common carp (EA012) isolate displayed a preference for common carp and salmon over sea trout and stickleback skin (Fig. 2C**Error! Reference source not found.**~~Error: Reference source not found~~). Lastly, the IZERs of the stickleback isolate (CF006) were significantly higher for salmon compared to sea trout or stickleback skin (Fig. 2D)**Error! Reference source not found.**~~Error: Reference source not found~~. Mean zoospore numbers in the 'test' and 'control' tubes for each isolate are presented in Table 4. In light of these results, the salmon EA001 and the sea trout EA016 isolates were selected for further *in vitro* induced zoospore encystment testing and *in vivo* challenge experiments (EA001 showing no preference for sea trout or salmon, compared to EA016 preferring sea trout skin).

When simultaneously presented with salmon and sea trout skin, zoospores of the salmon isolate (EA001) and the sea trout isolate (EA016) displayed consistently generalist and specialist induced zoospore encystment responses, respectively. For EA001, there were no significant differences in IZER for salmon and sea trout skin ($P$>0.05) (Fig. 3). Conversely, we confirmed the preference of EA016 for sea trout compared to salmon skin ($P$<0.0001) (Fig. 3). Mean zoospore numbers in ' salmon test', ‘sea trout test’ and 'control' tubes for each isolate are presented in Table 5.

### In vivo Saprolegnia parasitica *challenge infections*

Infection outcomes (asymptomatic, mild and cleared, severe) from the *S. parasitica* challenge infections revealed no evidence of isolate-specific host preference. Neither fish species (salmon, sea trout; Figs. 4A and B) nor fish population (North, South Tyne salmon; Figs. 4C and D) significantly affected fish infection outcomes for either parasite isolate (salmon isolate EA001, sea trout isolate EA016; GLMs; $df$=1, $P$>0.05). Infection outcomes were, however, significantly different between the two *S. parasitica* isolates (GLMs; $df$=1, $P$<0.0001); EA001 established infections in only 34% and 24% of salmon and sea trout respectively (Fig. 4A) in addition to 20% and 24% of North and South Tyne salmon (Fig. 4C). Conversely, EA016 successfully infected 100% of challenged fish (Figs. 4B and D).

Indications of host-preference were observed from the survival analysis; while there was no overall significant difference in the survival of salmon and sea trout challenged with the sea trout isolate (EA016) (log-rank test; $P$>0.05), there was a significant difference during the initial 48 h of the infection (Fig. 5A). At 24 h, sea trout survival was reduced to 84%, whereas none of the salmon died during this period (log-rank test; $P$<0.05; Fig. 5A). At 48 h, 60% of the sea trout were alive compared to 96% of the salmon (log-rank test; $P$<0.0001; Fig 5A). There was no significant difference in survival for North and South Tyne salmon challenged with the sea trout isolate (EA016) (log-rank test; $P$>0.05; Fig. 5B).

## Discussion

The current study is the first to investigate the host preference of *S. parasitica* at an isolate level. The *in vitro* induced zoospore encystment assays indicate that the salmon isolate (EA001) is a generalist, exhibiting a similar level of preference for three fish species, whereas the sea trout (EA016), carp (EA012) and stickleback (CF006) isolates are more specialised, showing a higher preference for one or two fish species. These *in vitro* results, however, were

not reflected in the *in vivo* challenge infections; no differences between salmon and sea trout infection outcomes were observed within the salmon isolate (EA001) or sea trout isolate (EA016) challenges. Infection outcomes between salmon populations (North and South Tyne) were also not significantly different for either isolate, suggesting host preference does not occur at a population level. Despite this, survival analysis revealed a significantly higher number of sea trout mortalities compared to salmon within 48 h of the sea trout isolate (EA016) challenge infection, which could be a potential indication of host preference/adaptation.

The phylogenetic analysis presented here offered no separation between the four isolates as they all possessed an identical ITS sequence and fell within Cluster 3 of the taxonomic system proposed by Sandoval-Sierra et al. (2014) alongside other isolates classified as *S. parasitica*. Despite the four *S. parasitica* isolates sharing the same ITS sequence – the DNA barcode traditionally used for *Saprolegnia* species identification – isolates were genetically distinct (confirmed via whole genome resequencing see Matthews 2020) and exhibited considerable differences in host preference and virulence, suggesting this region is not suitable for isolate discrimination.

Interestingly, all isolates examined here yielded a low induced zoospore encystment response to sticklebacks. Moreover, even the more 'specialist' isolates demonstrated a similar level of induced zoospore encystment towards fish from different families; the sea trout isolate (EA016), for instance, displayed a comparable level of preference for sea trout and carp (Family Salmonidae and Cyprinidae, respectively). Hence, reaffirming the postulation that *Saprolegnia* spp. are able to target a wide range of phylogenetically distant species. A potential criticism of the induced zoospore encystment assay is that homogenisation of the fish skin in solution would have destroyed its structure and biological components. However, El-Feki *et al.* (2003) employed the same methodology in their chemotaxis assay and found that fish skin

induced the highest chemotactic response compared to other fish tissue extracts including mucus, blood, and gills.

The drastically different levels of virulence displayed by the salmon (EA001) and sea trout (EA016) isolates in the challenge infections may be a reflection of different time in culture; EA001 was isolated 510 days before EA016. Maintaining pathogens (fungi, bacteria and viruses) on/in artificial culture media for extended periods can cause an attenuation of virulence (Druelle et al. 2008; Almaguer-Chávez et al. 2011; Ansari and Butt 2011). Passage through a susceptible fish host and subsequent re-isolation can restore virulence in *S. parasitica* cultures (Songe et al. 2014), there are, however contamination risks associated with re-culturing which could confound results.

Disparity between sea trout and salmon survival during the initial 48 h of the sea trout isolate (EA016) challenge infection could reflect the different induced zoospore encystment responses displayed by this isolate; if its zoospores are less attracted to salmon skin, as our induced zoospore encystment results would suggest, it may have taken longer to locate the salmon in comparison to the sea trout. It may also reflect subtle differences in the early stages of host-pathogen interaction. The initial attachment of *S. parasitica* zoospores to host cells is purportedly achieved via cell-binding proteins such as lectins (Jiang et al. 2013). In salmonids, this triggers a strong inflammatory response via the induction of proinflammatory cytokines and antimicrobial peptides (AMPs), in particular interleukin-1β1 (IL-1β1), IL-6 and tumour necrosis factor-α (TNF-α) (de Bruijn et al. 2012; Belmonte et al. 2014). Effector proteins and/or proteases then suppresses several constituents of the adaptive immune system by downregulating T-helper cell cytokines, antigen presentation machinery and immunoglobulins (Jiang et al. 2013; Belmonte et al. 2014). Subsequently host cells are attacked by a multitude of virulence factors in the form of proteases, lipases and lysing enzymes (Jiang et al. 2013). It may be that the salmon immune response was able to suppress the initial EA016 invasion,

however immune defences were eventually overcome and salmon survival plummeted from 96 to 60% between 48 and 60 h. Sequencing of the *S. parasitica* genome revealed the arsenal of virulence proteins employed by this pathogen are rapidly evolving due to co-evolution with the host (Jiang et al. 2013). Hence, isolates of *S. parasitica* could potentially target host species to which they have been more frequently exposed and consequently adapted to (Williams 1966; Kawecki and Ebert 2004; Savolainen et al. 2013). The sea trout isolate (EA016) examined here may be better adapted to sea trout, which is reflected in its induced zoospore encystment responses and ability to initiate rapid virulence and host mortality during the challenge experiment.

Overall, this study indicates that *S. parasitica* is a generalist with isolate variation in host preference. Uncovering the host preference of the key isolates within the UK could aid our understanding of disease outbreaks in the wild and fish management practises within aquaculture. For example, if the sea trout isolate (EA016) investigated here was present in a salmon aquaculture facility, knowledge of the 48 h lag in salmon mortality would prompt the application of treatments to boost immune function and potentially reduce mortalities. Furthermore, we demonstrate that the ITS region does not offer *S. parasitica* isolate separation and is not indicative of isolate severity.

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509 **Tables and Figures**

510

511 Table 1. Origin of fish used for induced zoospore encystment assays and challenge infections.

512

| Experiment | Fish Species | Life stage | Mean weight (g) + range | Mean standard length (mm) + range | Source | Date of arrival at Cardiff University |
|---|---|---|---|---|---|---|
| Induced zoospore encystment assays | Atlantic salmon (*Salmo salar*) | Juvenile | 17.4 (14.8-20.4) | 111.0 (104.6-117.8) | Kielder Salmon Hatchery, Hexham, Northumberland | June 2016 |
| | Sea trout (*Salmo trutta*) | Juvenile | 9.2 (7.8-10.2) | 92.3 (88.5-96.2) | | |
| | Common carp (*Cyprinus carpio*) | Juvenile | 41.41 (23.5-63.5) | 123.9 (103.8-138.2) | DC Freshwater Fish, Brookwood, Surrey | |
| | Three-spined stickleback (*Gasterosteus aculeatus*) | Adult | 1.7 (0.6-2.8) | 48.0 (43.9-57.2) | Roath Brook, Cardiff | |
| Challenge infection 1 | Atlantic salmon | Juvenile | 1.3 (0.7- 2) | 40.1 (34.9-48.2) | Kielder Salmon Hatchery, Hexham, Northumberland | July 2017 |
| | Sea trout | Juvenile | 1.2 (0.6-2.1) | 41.4 (32.9-48.1) | | |
| Challenge infection 2 | North Tyne Atlantic salmon | Juvenile | 2.7 (1 - 4.8) | 50.8 (36.9-64.9) | | |
| | South Tyne Atlantic salmon | Juvenile | 2.2 (1-4.1) | 49.1 (36.8-61.6) | | |

513

514 Table 2. Origin of *Saprolegnia parasitica* isolates used for the i*n vitro* induced zoospore
515 encystment assays. *Denotes isolates used in subsequent induced zoospore encystment assays
516 and challenge infections.

517

| Isolate | Host species | River/waterbody | Date isolated |
|---|---|---|---|
| EA001* | Atlantic Salmon (*Salmo salar*) | River Esk, Yorkshire, England. (54°26'59.1"N, 0°48'12.42"W) | 10/01/2015 |
| EA016* | Sea trout (*Salmo trutta*) | River Dart, Devon, England. (50°27'36.432"N, 3°41'42.144"W) | 03/06/2016 |
| EA012 | Common carp (*Cyprinus carpio*) | Lake near Romsey, Hampshire, England. (50°59'40.6"N, 1°34'46.7"W) | 22/03/2016 |
| CF006 | Three-spined stickleback (*Gasterosteus aculeatus*) | Roath Brook, Cardiff, Wales. (51°29'54.1572"N, 3°9'54.2484"W) | 15/07/2016 |

518

519 Table 3. Fish in the *Saprolegnia parasitica* experimental infections were categorised as either
520 asymptomatic, symptomatic (mild and cleared) or symptomatic (severe) of saprolegniasis
521 according to these symptoms.

| | Symptomatic | |
|---|---|---|
| Asymptomatic | Mild and cleared infection | Severe infection |
| • No signs of saprolegniasis | • Small tufts of mycelial growth on the body which were no longer present upon conclusion of the experiment | • Extensive mycelial body coverage<br>• Lethargy<br>• Respiratory distress<br>• Loss of equilibrium |

522

523

Table 4. Mean zoospore numbers of the salmon (EA001), sea trout (EA016), common carp (EA012) and three-spine stickleback (CF006) isolates in the 'test' and 'control' tubes of the *in vitro* induced zoospore encystment assays

| Isolate | Fish skin | Mean no. of zoospores in 'test' tubes | Mean no. of zoospores in 'control' tubes |
|---|---|---|---|
| EA001 | Atlantic Salmon (*Salmo salar*) | 5.25 | 0.5 |
| | Sea trout (*Salmo trutta*) | 5 | 0.35 |
| | Common carp (*Cyprinus carpio*) | 3.65 | 0.35 |
| | Three-spined stickleback (*Gasterosteus aculeatus*) | 0.85 | 0.35 |
| EA016 | Salmon | 2.6 | 0.45 |
| | Sea trout | 5.6 | 0.25 |
| | Common carp | 5.45 | 0.3 |
| | Three-spined stickleback | 1.7 | 0.2 |
| EA012 | Salmon | 2.55 | 0.2 |
| | Sea trout | 1.6 | 0.25 |
| | Common carp | 2.7 | 0.2 |
| | Three-spined stickleback | 0.85 | 0.35 |
| CF006 | Salmon | 3.05 | 0.2 |
| | Sea trout | 1.25 | 0.15 |
| | Common carp | 1.95 | 0.2 |
| | Three-spined stickleback | 2.5 | 0.45 |

Table 5. Mean zoospore numbers of the salmon (EA001) and sea trout (EA016) isolates in the 'salmon test', 'sea trout test' and 'control' tubes of the *in vitro* induced zoospore encystment assays

| Isolate | Mean no. of zoospores in 'salmon test' tubes | Mean no. of zoospores in 'sea trout test' tubes | Mean no. of zoospores in 'control' tubes |
|---|---|---|---|
| EA001 | 3.15 | 3.1 | 0.25 |
| EA016 | 3.15 | 8.3 | 0.3 |

Fig. 1. Induced zoospore encystment assays - experimental set-up. (A) Assay used to assess the induced zoospore encystment of four *Saprolegnia parasitica* isolates: EA001 (salmon), EA016 (sea trout), EA012 (common carp) and CF006 (three-spined stickleback) against a fish skin extract (from salmon, sea trout, common carp or three-spined stickleback) and PBS control. (B) Modified assay used to compare the induced zoospore encystment of isolates EA001 (salmon) and EA016 (sea trout) when simultaneously exposed to salmon and sea trout skin extracts alongside a PBS control.

Fig. 2. Induced zoospore encystment ratios of four *Saprolegnia parasitica* isolates: (A) Salmon isolate (EA001), (B) Sea trout isolate (EA016), (C) Common carp isolate (EA012) and (D) Three-spined stickleback isolate (CF006) against skin extracts from four fish species (salmon, sea trout, common carp and three-spined stickleback). Statistical significance displayed; $P<0.05$(*), $<0.01$ (**), $<0.001$ (***). Dots above and below the box and whisker plots signify outliers.

Fig. 3. Induced zoospore encystment ratios of the *Saprolegnia parasitica* zoospore isolates; salmon isolate (EA001) and sea trout isolate (EA016) when presented with a direct choice between salmon and sea trout skin extracts. Statistical significance displayed; $P<0.0001$ (***).

Fig. 4. Percentage of salmon and sea trout, and North and South Tyne salmon that were asymptomatic, mild and cleared and severely infected with saprolegniasis when challenged with the *Saprolegnia parasitica* salmon isolate (EA001; (A) and (C) respectively), and the sea trout isolate (EA016; (B) and (D) respectively).

Fig. 5. Survival plots showing percent survival (+95% confidence intervals) of (A) Salmon and sea trout, and (B) North and South Tyne salmon challenged with *Saprolegnia parasitica* sea trout isolate (EA016). Control fish (both species and populations) not challenged with *S. parasitica* displayed 100% survival (dashed line).